Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 19-45 are pending in the application, with 19, 26, 32, 39, 41 and 45 being the independent claims. Claims 19, 21, 24, 26-28, 30, 32, 36, 39 and 41- 45 have been amended to more particularly point out what Applicants regard as the invention and to make explicit that which was previously implicit in the claims. Support for these amended claims may be found throughout the specification, for example, at page 2, line 8 through page 3 line14; page 3, lines 4-9; page 4, lines 9-22; page 5, lines 10-24; page 8, line 31; page 9, line 32 through page 10, line 35; page 14, lines 14-17; page 15, lines 9-13; and at page 21, line 11. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objection to the Specification

The Examiner has stated that each letter of a trademark should be capitalized or otherwise the trademark should be demarcated with the appropriate symbol indicating its proprietary nature and be accompanied by generic terminology. (Paper No. 12, page 3.) Applicants have reviewed the specification for occurrences of trademarks and have amended the specification according to the Examiner's suggestion. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the objection.

Claim Objections

The Examiner has stated that claims 19-45 are objected to because "fluorescent" is consistently misspelled throughout the claims. (Paper No. 12, page 3.) Applicants have reviewed the specification for occurrences of this typographical error and have corrected the spelling of "fluorescent" in claims 19, 21, 26, 32, 41, 43, and 45. However, since claims 20, 22-25, 27-31,42 and 44 do not contain the word "fluorescent" these claims have not been amended.

The Examiner has also stated that claims 30 and 36 are objected to because "fibroblast" is misspelled. (*Id.*) Applicants have corrected this typographical error.

The Examiner has stated that claim 39 is objected to as being improper because "[a] claim that depends from a dependent claim should not be separated by any claim that does not also depend from said dependent claim." (*Id.*) Claim 39 has been amended to depend from claim 32.

Finally, the Examiner has stated that claim 45 is objected to because of the redundancy of "FACS" and "sorting." Applicants have eliminated the alleged redundancy in claim 45 by deleting the word "sorting."

In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the objections.

I. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 19-25 and 45 under 35 U.S.C. § 112, first paragraph, because the claims allegedly "[contain] subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or

with which it is most nearly connected, to make and/or use the invention." (Paper No. 12, page 4.) Applicants respectfully traverse the Examiner's rejection.

In regards to claims 19-25, the Examiner has stated that "[t]he specification does not teach a method for determining whether cells in a culture are undergoing apoptosis." (Paper No. 12, page 4.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 19 has been amended to recite "a method of determining the proportion of apoptotic cells *in a population of cells which have been transfected with a plasmid containing a DNA sequence of interest.*" (emphasis added.) The amendment renders moot the Examiner's rejection. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

In regards to claim 45, the Examiner has stated:

> It is not apparent that a cDNA molecule encoding a protein that induces the cell's apoptosis, once the cDNA molecule is transfected into the cell, can be isolated from transfected cells before the cDNA is degraded. Therefore, as the claims are now written, it is unclear that the invention can be practiced with a reasonable expectation of success, particularly in the absence of working exemplification of the claimed method.

(Paper No. 12, page 6.) Applicants respectfully disagree with the Examiner's rejection. Applicants note that the specification states that "the method according to the invention is capable of measuring the influence of gene expression *within* 24 to 48 hours. It is therefore possible to analyse and isolate cells while they are still alive." (Specification at page 15, lines 9-13, emphasis added.) During this time period, it would be possible to isolate the plasmid of interest without undue experimentation given that the cells are still alive. Thus, Applicants submit that there would be a reasonable expectation of success.

Furthermore, the Examiner has stated:

> The [sic] is an enormously diverse group of genes and proteins that have been found to affect apoptosis in cells, many of these gene's expression are regulated by exquisitely complex mechanisms and many of these proteins have markedly different functions. Therefore, the specification provides insufficient guidance to enable the skilled artisan to conduct expression and function studies to characterize the protein encoded by a cDNA molecule.

(Paper No. 12, pages 6-7.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 45 has been amended to delete step (I) which recited "characterizing the corresponding genes on the plasmids isolated and amplified in (H) by sequencing and conducting expression and function studies." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

II. Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 19-45 under 35 U.S.C.§112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully traverse the Examiner's rejection.

The Examiner has asserted that "[c]laims 19-25 and 45 are indefinite because claims 19 and 45 do not recite a positive process step that clearly relates back to the preamble of the claim." (Paper No. 12, section 10, page 7.) Contrary to the Examiner's assertion, claim 19 does, in fact, recite a positive process step. Specifically, claim 19 recites "thereby, determining the proportion of apoptotic cells in the transfected population." Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim

45 has been amended to recite a positive process step that relates back to the preamble of the claim. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that "[c]laims 19-25 are indefinite because claim 19 recites the phrase '[a] method of determining the proportion of apoptotic cells in a culture'." (*Id.*) The Examiner has stated that "[r]ecitation of the phrase renders the claim indefinite because it cannot be ascertained to which culture the claim refers."(*Id.*) As discussed earlier, claim 19 has been amended to recite "a method of determining the proportion of apoptotic cells in a population of cells which have been transfected with a plasmid containing a DNA sequence of interest." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that "[c]laims 19-40 and 45 are indefinite because claims 19, 26, 32 and 45 recite the term 'suitable nutrient medium'." The Examiner alleges that 'suitable' is a relative term which is not defined by the specification. (Paper No. 12, section 12, page 8.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 19, 26, 32 and 45 have been amended to delete the term "suitable" pursuant to the Examiner's suggestion. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has alleged that claims 19-25 are indefinite "because claim 19 recites the limitation 'the DNA sequence of interest' in line 6." (Paper No. 12, page 8.) Specifically, the Examiner is of the opinion that there is insufficient antecedent basis for the limitation. Claim 19 has been amended to provide sufficient antecedent basis for recitation of this

limitation in the claim. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 19-25 and 45 are indefinite "because claims 19, 26, 32, and 45 recite the limitation 'the apoptotic DNA fragments'." (*Id.*) Specifically, the Examiner is of the opinion that there is insufficient antecedent basis for the limitation. Claims 19, 26, 32 and 45 have been amended by deleting "the" in the limitation, according to the Examiner's suggestion. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 19-31 and 45 are indefinite "because claims 19, 26 and 45 recite the phrase 'so that the DNA sequence of interest or its expressed polypeptide exerts its potential activity'." (Paper No. 12, page 9.) Specifically, the Examiner has stated:

> Recitation of the phrase renders the claim indefinite because it is unclear if a potential activity can be exerted. If one defines "potential" as "existing in possibility or capable of development into actuality", then once an activity is exerted by the sequence of interest or the expressed polypeptide, the activity should no longer be described as potential. Because the specification does not provide a standard for ascertaining the requisite degree of suitability, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claims 19, 26, 32 and 45 to delete "potential" can obviate this rejection.

(Paper No. 12, page 9.) Applicants respectfully disagree with the Examiner's rejection.

However, solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 19, 26, 32 and 45 have been amended to delete the word "potential." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 19-40 and 45 are indefinite "because claims 19, 26, 32, and 45 recite the phrase 'so that *any* [fluorescent] protein expressed remains in the cells'." (Paper No. 12, page 9, emphasis in original.) The Examiner has stated:

> It is unclear that every molecule of the fluorescent protein (i.e., any one) present in the cell can be retained; some of the molecules may not be retained in the cells. Also, during the process of apoptosis, a cell forms apoptotic bodies, which are fragments of cells; therefore, the method is more likely to only retain fluorescent protein in the harvested cells that remain intact. Because of the uncertainty, the claim is indefinite and one of ordinary skill in the art would therefore not be reasonably apprised of the metes and bounds of the invention. Amending claims 19, 26, 32, and 45 to recite, for example, the phrase "so that most fluorescent protein expressed in the cells remaining intact is retained" could obviate this rejection.

(*Id.* at page 9.) Applicants respectfully disagree with the Examiner's rejection.

However, solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 19, 26, 32, and 45 have been amended to recite "so that a *measurable amount* of fluorescent protein expressed remains in the cells." (emphasis added.) Support for this amendment can be found in the specification at page 4, lines 13-22. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 19-40 and 45 are vague and indefinite "because claims 19, 26, 32, and 45 recite the phrase 'measuring the proportion of transfected cells by measuring total DNA content remaining subsequent to [step] (D)'." (Paper No. 12, page 10.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 19, 26, 32, and 45 have been amended to recite "measuring the proportion of the harvested cells from (D) containing a DNA content of less than 2 N and

thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made," according to the Examiner's suggestion. Support for this amendment can be found, for example, on page 5, lines 10-24 of the specification. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

In addition, the Examiner has asserted that claims 19-40 and 45 are vague and indefinite "because claims 19, 26, 32 and 45 recite the phrase 'measuring the proportion of transfected cells by quantitating the [fluorescent] marker protein contained in the cells harvested' in lines 14 and 15." (Paper No. 12, page 10.) Specifically, the Examiner has stated that "it cannot be ascertained how determining the quantity of the fluorescent marker protein contained in the harvested cells can be used to measure the proportion of transfected cells." (Paper No. 12, page 10.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 19, 26, 32, and 45 have been amended to recite "measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the plasmid containing a DNA sequence of interest" or "measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with said DNA sequence of interest." Support for this amendment can be found, for example, on page 4, lines 4-7 and page 19, lines 26-28, of the specification. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

In addition, the Examiner has asserted that claims 19-40 are indefinite "because claims 19, 26 and 32 recite the phrase 'comparing the values' and/or the phrase 'comparing the calculated proportion of apoptotic cells'." (Paper No. 12, page 10.) Specifically, the

Examiner stated "it cannot be ascertained how the claim requires the values to be compared." (*Id.*) Furthermore, the Examiner has asserted that "claims 19-25 are vague and indefinite because claim 19 recites the phrase 'determining the proportion of apoptotic cells in the transfected population' in line 16." (*Id.* at page 11.) Specifically, the Examiner stated "it cannot be ascertained how the claim requires the proportion of apoptotic cells in the transfected population is to be determined." (*Id.*)

Solely to expedite the allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 19, 26 and 32 have been amended to recite "measuring the proportion of the harvested cells from (D) containing a DNA content of less than 2 N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made" and "measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the sequence of interest." Support for this amendment can be found, for example, on page 2, line 8 through page 3 line14; page 4, lines 4-7; page 5, lines 10-24; and page 19, lines 26-28 of the specification. Applicant assert that, as amended, the claims reasonably apprize those skilled in the art as to what values are to be compared and as to how the proportion of apoptotic cells in the transfected population is to be determined. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that "claim 24 is vague and indefinite because the claim recites the limitation 'wherein the fixing and permeabilization in (D) is achieved with paraformaldehyde and ethanol, respectively' without reciting the specific conditions that are used to achieve the result." (Paper No. 12, page 11.) Solely to expedite allowance of the

claims, and not in acquiescence to the Examiner's rejection, claim 24 has been amended to recite "using", according to the Examiner's suggestion. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that "claims 26-31 are vague and indefinite because claim 26 recites the phrase 'determining whether the gene of interest affects the proportion of apoptotic cells in the transfected population'." (Paper No. 12, page 11.) Specifically, the Examiner has stated that "it cannot be ascertained how the claim requires the practitioner to determine whether the gene of interest affects the proportion of apoptotic cells in the transfected population." In response to an earlier rejection discussed above, claim 26 has been amended in order to more clearly and precisely describe the subject matter being claimed. Applicants submit that this amendment clarifies the claim and renders the Examiner's rejection moot. Thus, Applicants respectfully request that the rejection be withdrawn.

The Examiner has asserted that "claim 27-31 and 33-37 are indefinite because claims 27, 28, 33, and 34 recite the term 'particular to a tumor cell.'" (Paper No. 12, page 11.) Specifically, the Examiner stated that:

> Recitation of the term renders the claims indefinite because the term is not defined in the claim or appears not to be defined in the specification. It cannot be ascertained whether the claim requires the survival factor to be a distinguishing feature of only the single tumor cell to which the claim refers or in all tumor cells or in only some tumor cells, or not in non-tumor cells. In other words, it is unclear how the claim requires the survival factor to be particular to a tumor cell.

(Paper No. 12, pages 11-12.)

Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 27, 28, 33, and 34 have been amended to delete the allegedly indefinite phrase. As such, Applicants submit that the amendment clarifies the claims and that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claim 27, 33, and 39 are vague and indefinite because claims 27 and 33 recite the term "signal transmission molecule of a receptor." (Paper No. 12, page 12.) Specifically, the Examiner stated that "while the specification discloses example [sic] of molecules that are considered to be 'signal transmission molecule of a receptor,' because the term is not defined, it is still not apparent which molecules are encompassed by the claims, and which are not." (*Id.*) Applicants respectfully disagree with the Examiner's rejection.

Applicants submit that the term "signal transmission molecules" is well understood in the art and need not be defined in the specification. For example, Schenk P. *et al.*, *Biochim Biophys Acta 1449(1)*:1-24 (1999) [hereinafter "Shenk *et al.*"] review the modulation of intracellular transducing proteins. The abstract of Schenk *et al.* states:

> Cells can react to environmental changes by transduction of extracellular signals, to produce intracellular responses. Membrane-impermeable signal molecules are recognized by receptors, which are localized on the plasma membrane of the cell. Binding of a ligand can result in the stimulation of an intrinsic enzymatic activity of its receptor or the modulation of a transducing protein. The modulation of one or more intracellular transducing proteins can finally lead to the activation or inhibition of a so-called 'effector protein'. In many instances, this also results in altered gene expression. Phosphorylation by protein kinases is one of the most common and important regulatory mechanisms in signal transmission.

(*Id.* at pages 1-2.) Although the specification discloses several examples of signal transmission molecules of receptors, including "Ras, Raf, phosphoinositol (3) kinase (=PI(3)-kinase), MAP kniases, type B and type C protein kinase, phospholipase C, and also adapter molecules such as Shc, Grb-2"(Specification at page 11, lines 13-17), "a patent need not teach, and preferably omits, what is well known in the art." *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367 (Fed. Cir. 1986), cert. denied , 480 U.S. 947 (1987).

In view of the above, Applicants submit that one skilled in the art would clearly understand the metes and bounds of the term "signal transmission molecule of a receptor." However, solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 27, 28, 33, and 34 have been amended to recite "signal transduction molecule of a receptor." As such, Applicants submit that the amendment clarifies the claims and that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 32-40 are indefinite because claim 32 recites the step "selecting those test substances which exhibit a synergistic activity." (Paper No. 12, page 12.) Specifically, the Examiner has stated:

> [I]t cannot be determined to which activity of the test substances the claim refers. It cannot be determined whether the test substances are required to synergize with another test substance or alternatively, with some other undisclosed entity. Also, the claim does not recite a step in which a second, third, fourth, etc. test substance is used and so it is not clear to which other test substances the claim refers. Furthermore, since a test substance can have either pro- or anti-apoptotic activity, it is unclear what is meant by the term "exhibits synergistic activity."

(Paper No. 12, page 12.) Applicants respectfully disagree with the Examiner's rejection.

The specification discloses a method of screening substances for their effect on apoptosis and states that the screening process may be used "to find substances which exhibit synergism with the inhibition of the survival factor function" or "which increase the activity of apoptosis-inducing or -increasing molecules." (Specification at page 12, lines 30-31 and page 13, lines 12-14, respectively.) In addition, the specification provides examples of agents with which test substances may be screened to determine anti- or pro- apoptotic synergistic activity. For example, the specification states:

> In the screening, the test cells are incubated with known chemotherapeutic agents or with substances from a pool which are to be investigated for their potential chemotherapeutic activity and the effect on apoptosis is investigated by the method according to the invention. In particular a screening operation of this kind sets out to find any synergistic activity between the inhibition or absence of the survival factor function in tumour cells and known chemotherapeutic agents or potentially chemotherapeutically active substances.

(Specification at page 12, lines 17-27.)

In view of the above, Applicants submit that one skilled in the art would clearly understand the metes and bounds of the term "exhibits synergistic activity." However, solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 32 has been amended to delete the allegedly indefinite phrase. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has asserted that claims 32-40 are indefinite because claim 32 recites the phrase "determining whether the gene of interest affects the proportion of apoptotic cells in the transfected population." (Paper No. 12, pages 12-13.) Specifically, the Examiner has stated that "it cannot be ascertained how the claim requires the practitioner to determine

whether the gene of interest affects the proportion of apoptotic cells in the transfected population." (Paper No. 12, pages 12-13.) As noted earlier, claim 32 has been amended to recite "measuring the proportion of the harvested cells from (D) containing a DNA content of less than 2 N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made" and "measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the sequence of interest." Therefore, Applicants submit that this amendment renders this rejection moot and request its withdrawal.

The Examiner has asserted that "claims 41-44 are indefinite because claims 41 and 42 recite the term 'sufficient'."(Paper No. 12, page 13.) Specifically, the Examiner has stated that "it cannot be ascertained whether the claim requires each and every one of the reagents needed to perform the transfection to be contained in the kit or alternatively, a sufficient amount of one or more of the reagents to be contained in the kit." (Paper No. 12, page 13.) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claims 41 and 42 have been amended so that the claims no longer recite the term "sufficient." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has also asserted that "claims 41-44 are indefinite because claim 41 recites the limitations 'wherein: (A) the first container' in line 4, 'the [fluorescent] marker protein' in lines 5 and 6, 'the primary fixing solution' in line 9 and 'the secondary fixing/permeablizing solution' in line 10."(Paper No. 12, page 13.) Specifically, the Examiner stated that "there appears to be insufficient antecedent basis for recitation of these

limitations in the claim." (*Id.*) Claim 41 has been amended to provide sufficient antecedent basis for recitation of these limitations in the claim. Thus, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner has also asserted that "claims 41-44 are indefinite because claim 41 recites the term 'container means' in line 2 and then the term 'container' in lines 4, 5, 7, and 9-12."(Paper No. 12, page 13.) Specifically, the Examiner has stated that "it is unclear whether the 'container' is the same as a 'container means' to which the claim refers in the preamble." (*Id.*) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 41 has been amended so that the claim consistently recites the term "container means." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has also asserted that "claim 42 is indefinite because the claim recites the limitation 'wherein the transfection components'."(Paper No. 12, page 13.) Specifically, the Examiner stated that "there appears to be insufficient antecedent basis for recitation of this limitation in the claim from which claim 42 depends, *i.e.*, claim 41." (*Id.* at pages 13-14.) Claim 41 has been amended to provide sufficient antecedent basis for recitation of the limitation in the claim. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has also asserted that "claim 44 is indefinite because the claim recites the terms '2% paraformaldehyde' and '70% ethanol'."(Paper No. 12, page 14.) Specifically, the Examiner stated that "it cannot be determined if the concentrations are expressed at [sic] unit mass/unit volume or unit volume/unit volume." (*Id.*) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 44 has been amended

to recite "2% (w/v) paraformaldehyde." Support for this amendment can be found, for example, on page 8, line 31 of the specification. With respect to the phrase "70% ethanol", Applicants assert that it would be obvious to one skilled in the art that ethanol is prepared unit volume/unit volume as such is the common practice in the art. Applicants submit that the phrase is not indefinite and reasonably apprises one of ordinary skill in the art as to the metes and bounds of the claimed invention. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner has asserted that "claim 45 is indefinite because the claim recites the limitation 'the population of cells' in line 3." (Paper No. 12, page 14.) Specifically, the Examiner stated that "[t]here appears to be insufficient antecedent basis for recitation of the limitation in the claim." (*Id.*) Claim 45 has been amended to provide sufficient antecedent basis for recitation of the limitation in the claim. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has also asserted that "claim 45 is vague and indefinite because the claim recites the phrase 'single cells which deviate from an apoptosis background which is to be determined'."(Paper No. 12, page 14.) Specifically, the Examiner has stated:

> Recitation of the phrase renders the claim vague and indefinite because: (a) the term "apoptosis background" is not defined by the claim and it cannot be determined to what measurement the claim refers, (b) it cannot be determined when or how the claim requires "apoptosis background" to be measured, and (c) it cannot be determined how and to what extent the claim requires the single cells to deviate from an "apoptosis background", so it cannot be determined which cells the claim requires to be isolated.

(Paper No. 12, page 14.) Applicants respectfully disagree with the Examiner's rejection.

"Apoptotic background" is an apoptotic phenotype which, depending on the problem to be solved, is predefined in each individual experiment. One of ordinary skill in the art would recognize that the cells used in each experiment may already be undergoing apoptosis. Therefore, in order to determine whether or not a particular gene of interest has pro-apoptotic or anti-apoptotic activity, one of ordinary skill in the art would need to account for apoptosis which is part of the background, *i.e.*, not caused by the gene of interest. Without accounting for this apoptotic background, one of ordinary skill in the art would not be able to distinguish whether apoptosis is caused by the gene of interest or by some other factor. For example, Examples 1 and 3 in the specification used cells transfected with a control plasmid as a measure of background apoptosis. (Specification at page 17, line 32 through page 18, line 4 and page 20, line 22 through page 21, line 5.) In addition, the specification states:

> Another use for the method according to the invention is the expression cloning of genes which modulate apoptosis. For this, a complete cDNA expression library is transiently transfected into cells. The method according to the invention is capable of measuring the influence of gene expression within 24 to 48 hours. It is therefore possible to analyse and isolate cells while they are still alive. For this purpose the method is modified by using FACS sorting to isolate single cells which *deviate from an apoptosis background* which is to be determined. The plasmids transfected into these cells are isolated, amplified and selected in further transfection processes. Plasmids which contain an apoptosis-modulating gene are thus isolated. The corresponding genes are then characterized by sequencing and other studies of expression and function.

(Specification at page 15, lines 6-22, emphasis added.)

In view of the above, Applicants submit that one skilled in the art would clearly understand the metes and bounds of the term "apoptotic background." Accordingly, since the claims particularly point out and distinctly claim the subject matter which Applicants regard as their invention, withdrawal of the rejection is respectfully requested.

The Examiner has also asserted that "claim 45 is vague and indefinite because the claim recites the step 'isolating, amplifying and selecting the transfected plasmids in a further transfection process'."(Paper No. 12, page 14.) Specifically, the Examiner has stated that "(a) it cannot be determined to what further transfection process the claim refers and (b) it cannot be determined how the claim requires the transfected plasmids to be selected after isolating and amplifying the plasmids." (*Id.*) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 45 has been amended to recite "within 24 to 48 hours isolating and amplifying the cDNA from said isolated single cells." As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

The Examiner has also asserted that "claim 45 is vague and indefinite because the claim recites the step 'characterizing the corresponding genes on the plasmids isolated and amplified in [step] (H) by sequencing and conducting expression and function studies'."(Paper No. 12, page 15.) Specifically, the Examiner has stated:

> Recitation of the phrase renders the claim indefinite because: (a) it cannot be determined how the claim requires the corresponding genes on the plasmids to be characterized by sequencing and conducting expression and function studies, (b) it cannot be determined how or to what entity or entities the claim requires the genes on the plasmids to correspond, and (c) it cannot be determined which expression and

> function studies the claim would require the practitioner to conduct.

(*Id.*) Solely to expedite allowance of the claims, and not in acquiescence to the Examiner's rejection, claim 45 has been amended such that the step "characterizing the corresponding genes on the plasmids isolated and amplified in [step] (H) by sequencing and conducting expression and function studies" has been deleted. As such, Applicants submit that the rejection has been rendered moot and request its withdrawal.

III. Rejection under 35 U.S.C. § 103 (a) and 35 U.S.C. § 102 (a)

The Examiner has rejected claims 19, 21, 22, 23, 25 and 26 under 35 U.S.C.§102(a) as allegedly being anticipated by or, in the alternative, under 35 U.S.C.§103(a) as obvious over, Keane *et al., Proc. Annu. Meet. Am. Assoc. Cancer Res.* 38:Abstract No. 1148 (1997) [hereinafter "Keane *et al.* (1997)]. Applicants respectfully disagree.

The claimed invention is directed, generally, to methods of determining the proportion of cells undergoing apoptosis in a population of cells transfected with a gene of interest, whether a gene of interest has an effect on apoptosis of cells in a culture and the effect of a test substance on the pro- or anti-apoptotic activity of a gene of interest. Specifically, the claimed invention permits the *simultaneous* measurement of the fluorescence of marker protein and DNA content, preferably by FACS analysis. (Specification at page 5, lines 35-36 through page 6, lines 1-2.) The specification states

> With the methods available up till now it was not possible to combine these measurements as the demands made on the fixing with respect to measuring the fluorescent marker protein on the one hand and measuring the DNA content of the cells on the other hand were diametrically opposed and therefore seemed to be irreconcilable. The present invention

> makes it possible for the first time to carry out both measurements in the same cell population using a suitable fixing step.

(Specification at page 7, line 36 through page 8, lines 1-9.)

Keane *et al.* (1997) discuss methods for determining if the protein tyrosine phosphatase DEP-1 induces apoptosis in breast cancer cell lines in order to further define the mechanism by which DEP-1 overexpression inhibits cell growth. (Keane *et al.* (1997), page 171, lines 3-4.) This method does not utilize PI to simultaneously measure the proportion of apoptotic cells that have been co-transfected with a fluorescent marker protein. Rather, Keane *et al.* (1997) discuss a method that utilizes PI staining to assess the percentage of apoptotic DEP-1 transfected cells and indicate the results of this method can be confirmed by TUNEL assay. In addition, Keane *et al.* (1997) provide that, following TUNEL assay, DEP-1 transfected cells can be selected by co-transfecting cells with GFP and, following co-transfection, the percentage of apoptotic cells was determined. However, the method by which the percentage of apoptotic cells can be determined is not disclosed. The abstract states:

> By TUNEL assay the number of apoptotic cells in DEP-1 transfected cells was 6.5±2.0 whereas in pcDNA3 transfected cells it was 3.6±0.3 and in αDEP-1 cells it was 3.3±0.2. Transfected cells were then selected by co-transfection with green fluorescent protein. The percent of apoptotic cells in selected DEP-1 transfected cells was 25±0.5 and in pcDNA3 transfected cells was 10.7±2.0.

(Keane *et al.* (1997), page 171, lines 13-17.)

The Examiner has stated that:

> Permitting slight but insignificant variations, the steps, which are not explicitly taught by the reference, are understood to be necessary to perform the analysis by the method taught by

> the reference and are accordingly considered intrinsic to the method. While Keane, et al do not explicitly state that the harvested cells were fixed and permeabilized, so that apoptotic DNA fragments diffuse out of the cells before the proportion of apoptotic cells is measured, because the method used by Keane, et al to measure apoptosis was conventional in the art and the scientific basis for the steps of fixing and permeabilizing the cells was well understood, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have fixed and permeabilized the harvested cells before measuring the proportion of apoptotic cells.

(Paper No. 12, pages 16-17.)

The Examiner acknowledges that Keane et al. do not explicitly teach every element of the claims. However, the Examiner asserts that the missing steps are "intrinsic" to the method disclosed in Keane *et al.* The Examiner is essentially arguing that the deficiencies of Keane *et al.* are inherent to the method steps disclosed therein and thus Keane *et al.* anticipates and/or renders obvious the claimed invention.

"To establish inherence, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by *probabilities* or *possibilities*. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'" *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted); see M.P.E.P. § 2112 (emphasis added).

The Examiner has assumed that the use of PI to detect apoptosis and a fixing and permeabilizing step are inherent to the method employed by Keane *et al.* However, the Examiner himself acknowledges that the TUNEL method may have been employed by

Keane *et al.* instead of PI. (Paper No. 12, page 17.) Thus, the Examiner has acknowledged that the use of PI to detect apoptosis is not "necessarily present" in the Keane *et al.* disclosure. Further, even assuming, *arguendo*, that PI was used to detect apoptosis, Applicants note that a fixing step is not necessary for this method. Fixing cells would further prevent PI from entering the cells as "cell membrane integrity excludes propidium iodide from viable and apoptotic cells." (Sigma-Aldrich catalog, Application of product number P4170). Indeed, not all protocols that evaluate cell cycle using PI include a fixing step. (*See, e.g.*, Vermont Cancer Center Flow Cytometry Protocol, pages 2-3). Accordingly, neither the PI staining step for detecting apoptosis nor the fixing and permeabilizing step are "necessarily present" in the Keane *et al.* methods. Thus, the reference cannot inherently anticipate the claims.

The Examiner has also asserted that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use propidium iodide staining to measure the proportion of apoptotic cells in the selected population of transfected cells"and that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have fixed and permeabilized harvested cells before measuring the proportion of apoptotic cells." (Paper No. 12, pages 16-17.) Applicants respectfully disagree.

Even assuming, *arguendo*, that Keane *et al.* used PI to detect apoptosis, it would not have been "obvious" to fix and permeabilize the cells in the method in order that *simultaneous* measurement of apoptotic cells and transfected cells could occur. As evidence that there would not have been a motivation to combine nor a reasonable expectation of success in employing this method, Applicants direct the Examiner to Kalejta

et al., Cytometry 29:286-291 (1997) [hereinafter "Kalejta *et al.*"]. Kalejta *et al.* disclose that "the simultaneous detection of green fluorescent protein (GFP) and DNA content using propidium iodide (PI) by flow cytometry is made difficult because of the unique nature of these 2 fluorogenic reagents" (Kalejta *et al.*, page 286). Kalejta *et al.* also disclose:

> For PI to enter cells efficiently and to stain DNA quantitatively, the cells must first be permeabilized; ethanol treatment is a routine method to achieve this. However, this permeabilization step causes GFP, which is normally found in the cytoplasm, to leach out of the cells. Although the use of paraformaldehyde-based fixatives allows GFP to be maintained in cells and retain its fluorescence even after ethanol permeabilization, the protocol we commonly employ results in *inefficient PI staining and poor quality DNA histograms*.

(*Id.*, emphasis added.) As a remedy, Kalejta *et al.* teach a method that utilizes a GFP-fusion protein which localizes to and is retained by the cellular membrane upon ethanol permeabilization *without prior fixation.* (*Id.*, page 286.) Thus, Kalejta *et al.* provide evidence that, among other things, it would not have been obvious to use PI staining in conjunction with GFP staining as the claimed methods do.

In view of the above, Keane *et al.* neither inherently anticipate nor render obvious the claimed invention. Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

IV. Rejection under 35 U.S.C. § 102 (a)

The Examiner has rejected claims 19, 21, 22, 23 and 25-28 under 35 U.S.C.§102(a) as allegedly being anticipated by Kalejta *et al., Cytometry* 29:286-291 (December 1, 1997) [hereinafter "Kalejta *et al.*"]. Applicants respectfully traverse the rejection.

Pursuant to 37 C.F.R. § 1.55(a)(4), Applicants have previously submitted (on November 21, 2001) an English language translation of a non-English foreign application (DE 197 52 922.4, filed November 28, 1997) to which the instant application claims priority, as well as a statement that the translation of the certified copy is accurate. Accordingly, Applicants have overcome the date of the reference relied upon by the Examiner. Thus, Applicants respectfully request that the Examiner reconsider and withdrawal the rejection.

V. Rejections under 35 U.S.C. § 102 (e)

The Examiner has rejected claims 19, 21, 22, 23, 25 and 26 under 35 U.S.C.§102(e) as allegedly being anticipated by U.S. Patent No. 5,976,853 A [hereinafter "Guthridge *et al.*"]. Applicants respectfully disagree with the Examiner's rejection.

As discussed above, the claimed invention is directed, generally, to methods of determining the proportion of apoptotic cells in a culture, whether a gene of interest has an effect on apoptosis of cells in a culture and the effect of a test substance on the pro- or anti-apoptotic activity of a gene of interest. Specifically, the claimed invention permits the *simultaneous* measurement of the fluorescence of marker protein and DNA content, preferably by FACS analysis.

Guthridge *et al.* teaches methods of increasing or decreasing the activity of FIN13, a novel serine/threonine phosphatase to inhibit or enhance cellular proliferation and tissue growth (col. 1, lines 18-25). Specifically, Guthridge *et al.* teaches:

> HeLa cells were co-transfected with pcDNA3GFP and either (A) pRKERK2-HA, (B) βCMV, or (C) pRKFIN13. Sixty hours following transfection, cells were harvested and sorted by FACS. Recovered cells were fixed, stained with

> propidium iodide and analyzed for DNA content by flow cytometry.

(column 8, paragraph 3). This method does not simultaneously measure the proportion of apoptotic cells and cells that have been co-transfected with a fluorescent marker protein. Rather, Guthridge *et al.* teach a method that utilizes PI staining *after* the FACS sorting of GFP-positive cells.

In view of the above, Applicants submit that the Guthridge *et al.* reference relied upon by the Examiner does not teach each and every element of the claims. "To establish a *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggest ed by the prior art." (MPEP § 2143.03.) Accordingly, withdrawal of the rejection is respectfully requested.

VI. Rejections under 35 U.S.C. § 103 (a)

The Examiner has rejected claims 19-44 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Keane *et al.*, *Proc. Annu. Meet Am. Assoc. Cancer Res. 38*:A1148 (1997), [hereinafter "Keane *et al.* (1997)"] in view of Douglas *et al., J. of Immunological Methods 188*:219-228 (1995) [hereinafter "Douglas *et al.*"], Anderson *et al., Proc. National Acad. Sci. USA 93*:8508-8511 (1996) [hereinafter "Anderson *et al.*"], and Baker *et al., Nucleic Acids Research 25*:1950-1956 (1997) [hereinafter "Baker *et al.*"] and in further view of Keane *et al., Proc. Annu. Meet. Am. Assoc. Cancer Res. 37*:A299 (1996) [hereinafter "Keane *et al.* (1996)"] or in still further view of Sell *et al., Cancer Research 55*:303-306 (1995) [hereinafter "Sell *et al.*"] and Prager *et al., Journal of Clinical Investigation 90*:2117-2122 (1992) [hereinafter "Prager *et al.*"] or Chow *et al., Development 121*:4383-4393 (1995)

[hereinafter "Chow *et al.*"] or Strawn *et al. J. of Biol. Chem. 269*:21215-2122 (1994) [hereinafter "Strawn *et al.*"] or Trent *et al. EMBO Journal 15*:4497-4505 (1996) [hereinafter "Trent *et al.*"], as evidenced by the teachings of Chu *et al., Cytometry 36*:333-339 (1999) [hereinafter "Chu *et al.*"]. (Paper No. 12, page 20.) Applicants respectfully traverse this rejection.

In order to establish a *prima facie* case of obviousness, the Examiner must establish that there is some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. (*See, e.g.*, M.P.E.P. § 2143 at 2100-122 (Eighth Edition, August 2001).) Obviousness cannot be established by modifying the teachings of the prior art to produce the claimed invention unless there is some teaching, suggestion, or motivation to do so found either in the reference itself or in the knowledge generally available to one of ordinary skill in the art. *See In re Fine*, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988). In addition, the mere fact that a reference could conceivably be modified to make the claimed invention does not render the resultant modification obvious unless the prior art also suggests the desirability of that specific modification. *See In re Mills*, 16 USPQ2d 1430, 1432 (Fed. Cir. 1990). Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness.

The claimed invention is directed, generally, to methods of determining the proportion of cells undergoing apoptosis in a population of cells transfected with a gene of interest. These methods comprise transiently transfecting a population of mammalian cells with a plasmid containing a sequence of interest, co-transfecting these cells with a plasmid containing DNA coding for a fluorescent protein and culturing the cells in the presence or

absence of a test substance such that the DNA sequence of interest or its expressed polypeptide exerts its potential activity on apoptosis, harvesting, fixing, and permeabilizing the cells, and *simultaneously* measuring total DNA content and quantitating the fluorescent marker protein. These values are compared, thereby determining the proportion of apoptotic cells in the transfected cell population.

Keane *et al.* (1997) disclose methods for determining if the protein tyrosine phosphatase DEP-1 induces apoptosis in breast cancer cell lines in order to define the mechanism by which DEP-1 overexpression inhibits cell growth. (Keane *et al.* (1997), page 171, lines 3-4.) Keane *et al.* do not utilize PI to measure the proportion of apoptotic cells that have been co-transfected with a fluorescent marker protein. Rather, Keane *et al.* (1997) teach a method that utilizes PI staining to assess the percentage of apoptotic DEP-1 transfected cells and that the results of this method can be confirmed by TUNEL assay. In addition, Keane *et al.* (1997) teach that, following TUNEL assay, DEP-1 transfected cells can be selected by co-transfecting cells with GFP and, *following* co-transfection, the percentage of apoptotic cells can be determined. The method by which the percentage of apoptotic cells can be determined is not disclosed. In addition, Keane *et al.* do not teach the simultaneous measurement of the fluorescence of marker protein and DNA content. Thus, Keane *et al.* do not teach or suggest every element of the claims.

Douglas *et al.* discuss a method for measuring apoptosis of phenotypically distinct cell populations or subsets in a mixed lymphocyte population. Specifically, a gentle detergent permeabilization and paraformaldehyde fixation procedure was combined with PI staining. However, Douglas *et al.* do not teach nor suggest the simultaneous measurement

of the fluorescence of marker protein and DNA content. Thus, Douglas *et al.* do not cure the defects of Keane *et al.*

Anderson *et al.* disclose a method for GFP mutagenesis and transient transfection of mutagenized GFP into NIH3T3 cells. Specifically, GFP variants were generated that have improved stability and increased fluorescence as compared to wild-type GFP. Two of the mutant GFPs generated by the Anderson *et al.* method also offer the advantage that they are spectrally distinguishable from one another by FACS, permitting quantitative analysis of two different genes within a single cell. However, Anderson *et al.* do not teach nor suggest utilizing PI to measure the proportion of apoptotic cells that have been co-transfected with a fluorescent marker protein nor the simultaneous measurement of the fluorescence of marker protein and DNA content. Therefore, the teaching by Anderson *et al.* does not cure the defects of Keane *et al.* and Douglas *et al.*

Baker *et al.* discuss a method for using bacterial artificial chromosomes (BACs) for the efficient transfection of large DNAs and stable gene expression in non-dividing cell cultures. Specifically, these BACs bear a marker gene for GFP, which can be visualized by fluorescence microscopy. (*See* Figure 2B.) Baker *et al.* state that "this method will be useful in screening BAC molecules for gene functions in eukaryotic cells" and that "other possible applications include the study of higher order chromatin structure, and its role in gene regulation, as well as the study of regulatory elements which, in their natural state, may be spread over hundreds of kilobases of the genome." (Baker *et al.*, page 1956.) The Examiner has stated that:

> One would have been motivated to fix the cells before analysis so as to prevent GFP from leaching out of the cells after permeabilization and one would have been motivated to permeabilize the cells because it was already established that

> permeabilization of the cells enables small apoptotic DNA fragments to diffuse out of the cells, thus creating the signature hypodiploid state, which distinguishes the apoptotic cell from the non-apoptotic cell.

(Paper No. 12, page 22.) Applicants respectfully disagree. As taught by the specification, up until the time of the instant invention,

> [i]t was not possible to combine these measurements as the demands made on the fixing with respect to measuring the fluorescent marker protein on the one hand and measuring the DNA content of the cells on the other hand were diametrically opposed and therefore seemed to be irreconcilable. The present invention makes it possible for the first time to carry out both measurements in the same cell population using a suitable fixing step.

(Specification at page 8, lines 1-9.)

Baker *et al.* do not teach nor suggest utilizing PI to measure the proportion of apoptotic cells that have been co-transfected with a fluorescent marker protein nor do they teach or suggest the simultaneous measurement of the fluorescence of marker protein and DNA content. Therefore, the teaching by Baker *et al.* does not cure the defects of Keane *et al.* (1997), Douglas *et al.*, and Anderson *et al.* Accordingly, the claimed invention would not have been obvious over Keane *et al.* (1997) in view of Douglas *et al.*, Anderson *et al.*, and Baker *et al.*

The Examiner has stated that "one would have had a reasonable expectation of success in practicing the method for determining the proportion of apoptotic cells transfected with an expression vector encoding GFP, as evidenced by the teachings of Chu, et al" (Paper No. 12, page 22). Applicants respectfully disagree.

As discussed above, pursuant to 37 C.F.R. § 1.55(a)(4), Applicants have previously submitted (on November 21, 2001) an English language translation of a non-English foreign

application (DE 197 52 922.4, filed November 28, 1997) to which the instant application claims priority, as well as a statement that the translation of the certified copy is accurate. Accordingly, Chu *et al.*, published in 1999, is improperly cited as prior art against the claimed invention.

Keane *et al.* (1996) discuss a method of inhibiting cell growth. Specifically, DEP-1 is transfected into cancer cell lines under the control of either a CMV promoter or a zinc-inducible metallothionein promoter. However, Keane *et al.* (1996) do not suggest that DEP-1 expression induces apoptosis. In addition, Keane *et al.* (1996) do not suggest the desirability or feasibility of modifying this method by transiently co-transfecting cells with a plasmid containing DNA coding for a fluorescent protein. Furthermore, Keane *et al.* (1996) do not suggest the simultaneous measurement of the fluorescence of marker protein and DNA content. Therefore, Keane *et al.* (1996) do not cure the defects of Keane *et al.* (1997), Douglas *et al.*, Anderson *et al.* and Baker *et al.*

Sell *et al.* discuss a method of determining if a sequence of interest, specifically the IGF-I receptor, inhibits apoptosis. First, BALB/c3T3 cells are transfected with IGF-I receptor and are exposed to etoposide, a toposmerase I inhibitor which is known to induce apoptosis. Next, cells are cultured in the presence or absence of IGF-I. Finally, cells are washed in PBS, fixed with ethanol, resuspended in PBS, stained with PI and evaluated by FACs. (Baker *et al.*, page 303, paragraph 7.) The absence of a pre-G1 apoptotic peak in cells cultured in the presence of IGF-I demonstrates that IGF-I receptor inhibits apoptosis. (Baker *et al.*, page 304, Figure 3A.) Sell *et al.* do not suggest the desirability or feasibility of modifying this method by transiently co-transfecting cells with a plasmid containing DNA coding for a fluorescent protein nor do they teach or suggest the simultaneous

measurement of the fluorescence of marker protein and DNA content. Therefore, this teaching by Sell *et al.* does not cure the defects of Keane *et al.* (1996), Keane *et al.* (1997), Douglas *et al.*, Anderson *et al.*, Baker *et al.* and Keane *et al.* (1996).

Prager *et al.* disclose a method of evaluating ligand-mediated endogenous rat IGF-I signaling. Specifically, mutant IGF-I receptors were tansfected into GC rat pituitary cells, evaluated for their ability to form nonfunctional human/rat hybrid receptors and to mediate dominant negative loss of function on normal receptor responses, *i.e.* IGF-I receptor half-life, autophosphorylation, phosphorylation of endogenous substrate, pituitary signaling, and growth hormone secretion. However, Prager *et al.* do not suggest testing the effect of mutant IGF-I receptors on apoptotic activity. Furthermore, Prager *et al.* do not suggest the desirability or the feasibility of co-transfecting GC cells with a fluorescent marker protein nor the simultaneous measurement of the fluorescence of marker protein and DNA content. Therefore, Prager *et al.* do not cure the defects of Sell *et al.*, Keane *et al.* (1996), Keane *et al.* (1997), Douglas *et al.*, Anderson *et al.* and Baker *et al.*

Chow *et al.* discuss a method of evaluating the role of FGF in fibre cell differentiation and lens development. Specifically, Chow *et al.* generated transgenic mice that express the dominant negative form of the murine FGF receptor-1 ($FGFR^{DN}$) and evaluated lens fibre cells differentiation. Chow *et al.* determined, by morphological and biochemical analysis, that fibre cells in the central region of the lens of $FGFR^{DN}$ mice apoptosed, indicating that these cells depend on FGF for their survival. However, Chow *et al.* do not suggest the desirability or the feasibility of co-transfecting lens fibre cells with a fluorescent marker protein, nor the desirability or the feasibility of simultaneously measuring the fluorescence of marker protein and DNA content. Therefore, this teaching by

Chow *et al.* does not cure the defects of Prager *et al.*, Sell *et al.*, Keane *et al.* (1996), Keane *et al.* (1997), Douglas *et al.*, Anderson *et al.* and Baker *et al.*

Trent *et al.* disclose a method of determining the role that the H-*ras* onocogene plays in TNF-induced apoptosis. Specifically, the method of Trent *et al.* comprises introducing two different molecular antagonists of H-*ras*, either the rap1A tumor suppressor gene or the dominant-negative rasN17 gene, into L929 TNF-sensitive fibroblasts. These H-ras antagonists inhibited TNF-induced apoptosis, demonstrating that Ras activation is required for TNF-induced apoptosis in mouse fibroblasts. However, Trent *et al.* do not suggest the desirability or the feasibility of co-transfecting mouse fibroblasts with a fluorescent marker protein nor the desirability or the feasibility of simultaneously measuring the fluorescence of marker protein and DNA content. Therefore, this teaching by Trent *et al.* does not cure the defects of Chow *et al.*, Prager *et al.*, Sell *et al.*, Keane *et al.* (1996), Keane *et al.* (1997), Douglas *et al.*, Anderson *et al.*, and Baker *et al.*

The Examiner has stated that it would have been *prima facie* obvious to one of ordinary skill in the art to make and use a kit comprising the reagents in view of the cited references. (Paper No. 12, page 25.) As discussed above, the claimed methods are not obvious in view of the cited references. In addition, Applicants submit that the cited references do not suggest the desirability of combining the references to obtain the claimed invention, and thus fail to render the claimed invention obvious. Accordingly, the Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

The specification is amended as follows:

At page 17, line 28 through page 18, line 17

About 80,000 cells were seeded into a 6 cm well in a 6-well culture dish and cultivated in DMEM, supplemented with 10 % FCS (v/v), 2 mM glutamine, 100 International Units of penicillin and 100 µg/ml of streptomycin, until 70 % confluence was achieved. The cells were transfected with 1 µg of a plasmid coding for eGFP ("enhanced GFP"; pEGFP-C1; Clontech) together with 1 µg of a control plasmid (pMEX; (22)), a pCMV plasmid containing the pro-apoptotic Adenovirus gene E1A or a pCMV plasmid containing the anti-apoptotic Adenovirus gene E1B-19K (17, 18) using 10 µl of [LipofectAMINE] LipofectAMINE ™ (GIBCO-BRL) in accordance with the manufacturer's recommendations. After the transfection the cells were left to stand for 16 h in complete medium, then the cells were either left untreated or treated for a further 16 h with an apoptotic stimulus (800 ng/ml of staurosporin; Sigma) (19, 20). 32 h after the transfection the detached cells were combined with trypsinised, adherent cells, washed twice with 4 ml of PBS and fixed at ambient temperature for 30 min (2% paraformaldehyde, 100 mM NaCl, 300 mM saccharose, 3 mM $MgCl_2$, 1 mM EGTA (ethyleneglycol-bis-(2-aminoethyl)-tetraacetic acid), 10 mM PIPES (piperazine-N_1N^1-bis[z-ethanesulphonic acid]) pH 6.8). Then they were washed twice with 4 ml of PBS and fixed for 14 h in ice-cold 70 % EtOH.

At page 18, line 19 through page 19, line 29

After the fixing, the cells were washed twice with 4 ml of PBS and divided up. One half of the sample was treated with RNase A (Sigma, St. Louis, USA) (50 µg/ml) in PBS for 30 min, washed twice with 4 ml of PBS and, 30 min before the FACS analysis, stained with propidium iodide in PBS (PI; 50 µg/ml; Sigma, St. Louis, USA). The other half of the sample was incubated for 1 hour at 37°C with 50 µl of TdT reaction mixture (terminal deoxynucleotidyl transferase; Boehringer Mannheim; 200 mM potassium cacodylate, 25 mM of Tris-HCl, pH 6.6, 0.25 mg/ml bovine serum albumen, 1 mM CoCl2; 0.25 nmol [FluoroLink] FluoroLink™ Cy5AP3-dCTP [Amersham], 12.5 units of TdT), washed twice with 4 ml of PBS, treated with RNase in PBS (50 µg/ml) for 30 min, washed twice with 4 ml HBS (from this step onwards HBS was used instead of PBS because DAPI has a tendency to produce microprecipitates in PBS), stained with DAPI in HBS (10 µg/ml; Sigma) for 20 min and analysed using a Becton Dickinson [FACS Vantage] FACSVantage™ apparatus. The FACS analysis of the PI-stained cells was carried out with a Becton Dickinson FACScan apparatus fitted with a so-called "doublet discrimination module", with which cell aggregates are discriminated by calculating the pulse width and pulse width. The results of the tests are shown in Fig.1. Fig. 1A shows the number of apoptotic βHC 13T tumour cells (% apoptosis) in the entire eGFP-positive cell population. The black bars indicate the determination of the sub-2N DNA content (GFP/PI); the white bars indicate the incorporation of fluorescent Cy5AP3-dCTP during the TdT reaction (GFP/TUNEL). The addition of staurosporin is shown. An excitation wavelength of 488 nm was used for eGFP and PI, an excitation wavelength of 647 nm was used for Cy5 and

UV of a wavelength range of 51 - 364 nm was used for DAPI. The emission fluorescence was collected using a 530/20 nm narrow band filter for eGFP, a 610 nm blocking filter for PI, a 675/20 nm narrow band filter for Cy5 and a 424/44 narrow band filter for DAPI. Doublets were excluded by means of pulsed processing. eGFP-expressing cells were selected and analysed for Cy5- or PI-fluorescence. The data were analysed using [CELLQuest] CELLQuest ™ software (Becton Dickinson). Each bar represents the average of 3 transfections, standard deviations are indicated by error bars. Each measurement comprised 40,000 total events, selected according to size and single cells. The transfection efficiency was 20-30 %.

At page 20, lines 6-19

In this Example an untransformed rat fibroblast cell line designated Rat1A was used. The cells were transiently transfected using either [LipofectAMINE] LipofectAMINE ™, as desribed in Example 1, or polyethyleneimine (PEI 2000)-DNA-Adenovirus complexes (WO 93/07283). Moreover, regarding the treatment of the cells and determination of apoptosis, using the process according to the invention on the one hand and the TUNEL method on the other hand, the procedure was exactly as described in Example 1. A comparison of the different transfection methods and methods of measuring apoptosis is shown in the Table. Each value represents the average of 3 transfections; the standard deviation is given (s.d.).

The claims are amended as follows:

19. (Once Amended) A method of determining the proportion of apoptotic cells in a population of cells which have been transfected with a plasmid containing a DNA sequence of interest [in a culture], comprising:

(A) transiently transfecting a population of mammalian cells with a plasmid containing a DNA sequence of interest;

(B) co-[transfecting] expressing in the population of cells [with a plasmid containing DNA coding for] a [flourescent] fluorescent marker protein;

(C) culturing the cells in a [suitable] nutrient medium so that [the] said DNA sequence of interest or its expressed polypeptide exerts its [potential] activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that [any flourescent] a measurable amount of fluorescent protein expressed remains in the cells, while [the] apoptotic DNA fragments diffuse out;

(E) measuring the proportion of [apoptotic cells by measuring]

the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made [total DNA content remaining subsequent to (D)];

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing [transfected cells by quantitating the flourescent] fluorescent marker protein [contained in the cells harvested in (D)] and thereby determining the proportion of the harvested cells transfected with the plasmid containing a DNA sequence of interest; and

(G) comparing the values obtained in (E) and (F);

thereby, determining the proportion of apoptotic cells in the transfected population.

21. (Once Amended) The method of claim 19, wherein the flourescent marker protein in (B) is Green [Flourescent] Fluorescent Protein.

24. (Once Amended) The method of claim 19, wherein the fixing and permeabilization in (D) is achieved [with] using paraformaldehyde and ethanol, respectively.

26. (Once Amended) A method of determining whether a [gene] DNA sequence of interest has an effect on apoptosis of cells in a culture, comprising:

(A) transiently transfecting a population of mammalian cells with a plasmid containing a DNA sequence of interest, thereby obtaining population X; and transiently transfecting another population of the same cells with a control plasmid, thereby obtaining a population Y;

(B) co-[transfecting] expressing in the population of cells [with a plasmid containing DNA coding for] a [flourescent] fluorescent marker protein;

(C) culturing the cells in a [suitable] nutrient medium so that [the] said DNA sequence of interest or its expressed polypeptide exerts its potential activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that [any flourescent] a measurable amount of fluorescent protein expressed remains in the cells, while [the] apoptotic DNA fragments diffuse out;

(E) measuring the proportion of [apoptotic cells by measuring] the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made [total DNA content remaining subsequent to (D)];

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing [transfected cells by quantitating the flourescent] fluorescent marker protein [contained in the cells harvested in (D)] and thereby determining the proportion of the harvested cells transfected with said DNA sequence of interest; and

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells; and

(H) comparing the calculated proportion of apoptotic cells obtained in (G) for the populations X and Y [,]

thereby, determining whether [the gene] said DNA sequence of interest affects the proportion of apoptotic cells in the transfected population.

27. (Once Amended) The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative signal [transmission] transduction molecule of a receptor for a survival factor [particular to a tumor cell].

28. (Once Amended) The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative receptor for a survival factor [particular to a tumor cell].

29. (Twice Amended) The method of claim 28, wherein the dominant negative receptor is the [Fibrobalst] Fibroblast Growth Factor (FGF) receptor.

32. (Once Amended) A method of determining the effect of a test substance on the pro- or anti-apoptotic activity of a [gene] DNA sequence of interest, comprising:

(A) transiently transfecting two populations of mammalian cells with an identical plasmid containing a [gene] DNA sequence of interest;

(B) co-[transfecting] expressing in the population of cells [with a plasmid containing DNA coding for] a [flourescent] fluorescent marker protein;

(C) culturing one population of transfected cells in a suitable nutrient media containing a test substance, thereby obtaining a population X; and incubating the other population of transfected cells in a [suitable] medium lacking the test substance, thereby obtaining a population Y;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that [any flourescent] a measurable amount of fluorescent protein expressed remains in the cells, while [the] apoptotic DNA fragments diffuse out;

(E) measuring the proportion of [apoptotic cells by measuring] the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made [total DNA content remaining subsequent to (D)];

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing [transfected cells by quantitating the flourescent] fluorescent marker protein [contained in the cells harvested in (D)] and thereby determining the proportion of the harvested cells transfected with the plasmid containing said DNA sequence of interest; and

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells; and

(H) comparing the calculated proportion of apoptotic cells obtained in (G) for the populations X and Y; [and]

[(I) selecting those test substances which exhibit a synergistic activity;]

thereby, determining whether the test substance has an effect on the [proportion of apoptotic cells transfected with] pro- or anti-apoptotic activity of [gene] said DNA sequence of interest.

33. (Once Amended) The method of claim 32, wherein the mammalian cells in (A) are tumor cells; the gene of interest in (A) is a dominant negative version of a signal [transmission] transduction molecules of a receptor for a survival factor particular to a tumor cell, which causes the inhibition or absence of a survival factor function; and the test substance exhibits activity synergistic with the inhibition or absence of the survival factor function.

36. (Twice Amended) The method of claim 34, wherein the receptor for a survival factor is the [Fibrobalst] Fibroblast Growth Factor (FGF) receptor.

39. (Once Amended) The method of claim [33] 32, wherein the test substance in (C) acts synergistically with chemotherapy.

41. (Once Amended) A kit for the simultaneous measurement of the fluorescence of marker protein and DNA content in order to determine [determining] the proportion of apoptotic cells in a culture which comprises a carrier means being compartmentalized to receive in close confinement one or more container means wherein:

(A) [the] a first container means holds one or more components [sufficient] suitable for transfection;

(B) another container means holds a plasmid containing the sequence coding for [the flourescent] a fluorescent marker protein;

(C) another container means holds an empty vector for inserting the DNA sequence of interest and for control measurements;

(D) another container means holds [the] a primary fixing solution;

(E) another container means holds [the] a secondary fixing/permeabilizing solution;

(F) another container means holds washing solution(s); and

(G) a final container means holds a DNA-binding stain.

42. (Once Amended) The kit in claim 41, wherein the [transfection components] one or more components suitable for transfection in (A) [are sufficient to] can achieve receptor-mediated endocytosis using polyethyleneimine and psoralen/UV-inactivated Adenovirus.

43. (Once Amended) The kit in claim 41, wherein the flourescent marker protein in (B) is Green [Flourescent] Fluorescent Protein.

44. (Once Amended) The kit in claim 41, wherein the primary fixing solution in [step] (D) is 2% (w/v) paraformaldehyde and the secondary fixing/permeabilization solution in (E) is 70% ethanol.

45. (Once Amended) A method of [expression cloning of] identifying a gene which modulates apoptosis comprising:

(A) transiently transfecting a [complete] cDNA expression library into a population of cells;

(B) co-[transfecting the] expressing in said population of cells [with a plasmid containing DNA coding for] a [flourescent] fluorescent marker protein;

(C) culturing the cells in a [suitable] nutrient medium so that [the] a DNA sequence of interest or its expressed polypeptide exerts its [potential] activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that [any flourescent] a measurable amount of fluorescent protein expressed remains in the cells, while [the] apoptotic DNA fragments diffuse out;

(E) measuring the proportion of [apoptotic cells by measuring] the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made [total DNA content remaining subsequent to (D)];

(F) also, simultanesously measuring the proportion of the harvested cells in (D) containing [transfected cells by quantitating the flourescent] fluorescent marker protein

[contained in the cells harvested in (D)] and thereby determining the proportion of th harvested cells transfected with said DNA sequence of interest; and

(G) using FACS [sorting] to isolate [single] cells which [deviate] differ from an apoptosis background which is to be determined; and

(H) isolating[,] and amplifying [and] the [selecting] [transfected plasmids] cDNA from said isolated cells [in a further transfection process;]

[(I) characterizing the corresponding genes on the plasmids isolated and amplified in (H) by sequencing and conducting expression and function studies;]

thereby identifying a gene which modulates apoptosis.